Volume 192, number 2 FEBS 3083 November 1985

Molecular forms of glucagon-like peptides in man

S.K. George, L.O. Uttenthal, M. Ghiglione and S.R. Bloom*

Department of Medicine, Royal Postgraduate Medical School, Du Cane Road, London W12 0HS, England

Received 11 September 1985

Molecular forms of the glucagon-like peptides (GLP) encoded by the human preproglucagon gene were analysed by chromatography combined with specific radioimmunoassays to the synthetic peptides. Whereas extracts of human pancreas and a glucagonoma contained a large proglucagon cleavage product possessing both GLP-1 and GLP-2 immunoreactivities, extracts of human intestine contained products corresponding to free GLP-1 and a small amount of chromatographically distinct GLP-2 immunoreactivity. It is concluded that post-translational processing of proglucagon differs in pancreas and intestine, so that the C-terminal portion of the molecule is cleaved to liberate free GLP-1 in the intestine. Further processing or degradation results in loss especially of GLP-2 immunoreactivity.

Glucagon-like peptide *Proglucagon* *Post-translational processing* *Pancreas* *Intestine*

1. INTRODUCTION

The human preproglucagon gene encodes 2 further glucagon-like peptide sequences, GLP-1 and GLP-2, in the C-terminal portion of proglucagon [1]. These sequences are well conserved between mammalian species [2], suggesting that they have a significant biological role, but their post-translational cleavage in human pancreas and intestine has not been fully described. We have previously shown, by the use of an antiserum raised to a synthetic N-terminal fragment of GLP-1, that relatively little free GLP-1 is produced in the human pancreas [3]. Here, we have used antisera raised against synthetic entire sequences of GLP-1 and GLP-2 to investigate further their molecular forms in the human pancreas, a pancreatic glucagon-secreting tumour, and the human intestine.

2. MATERIALS AND METHODS

2.1. *Materials*

Synthetic GLP-1 was purchased from Bachem, CA, USA; synthetic des-Gly^{37}-GLP-1 amide and human GLP-2 from Peninsula Laboratories, CA, USA; and bovine serum albumin (BSA, Cohn fraction V) from Sigma. Other reagents were of analytical grade.

2.2. *Radioimmunoassays (RIA)*

GLP-1 and GLP-2 were conjugated to BSA in a molar ratio (peptide:protein) of 4:1 by bisdiazobenzidine and glutaraldehyde methods [4], respectively. Four rabbits were immunised with each conjugate in Freund's complete adjuvant, and boosted at monthly intervals. Serum collected after the second and subsequent boosts were used for RIA. GLP-1 and GLP-2 were trace-iodinated with carrier-free Na^{125}I [5] and purified by reverse-phase, high-pressure liquid chromatography (HPLC) as described in [3], under isocratic elution with water:acetonitrile:trifluoroacetic acid (574:425:1, v/v). Specific activities of the GLP-1 and GLP-2 tracers, as estimated by self-displacement from the appropriate antibody, were 81 and 20 Bq/fmol respectively, GLP-2 presumably being labelled on the sole His^{1} residue. RIA methods [3] and RIA for glucagon-like immunoreactivity (GLI) [6] were as described, using 0.2 M glycine

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V. (Biomedical Division)

sodium buffer, pH 8.8, containing 0.05% (w/v) sodium azide and 1% (w/v) BSA. The detection limit (95% confidence) for GLP-1 was 1.25 fmol/ml, and 5 fmol/ml for GLP-2. Cross-reactivities of glucagon and GLP-2 in the GLP-1 assay were 0.3 and <0.01% respectively, of glucagon and GLP-1 in the GLP-2 assay <0.01%, and of GLP-1 and GLP-2 in the GLI assay <0.01%. There were no significant cross-reactivities with other pancreatic or intestinal regulatory peptides.

2.3. *Tissues and extraction*

Five specimens of histologically normal human pancreas, specimens of human intestine and a human pancreatic glucagonoma removed at surgery were immediately frozen in liquid nitrogen and stored at −20°C before extraction for 15 min in 0.5 M acetic acid (10 ml/g wet wt tissue) at 100°C.

2.4. *Chromatography*

Gel filtration was performed at 4°C on a column (80 × 1.4 cm diameter bed) of Sephadex G-50 superfine eluted with assay buffer at a flow rate of 5 ml/h. Elution positions were expressed in terms of K_{av} [7] and dextran blue (M_r 2 × 10^6, K_{av} 0), horse heart cytochrome *c* (M_r 12384, K_{av} 0.17) and $Na^{125}I$ (K_{av} 1.0) were used as markers. HPLC was performed as described [3], with linear gradients of water:acetonitrile:trifluoroacetic acid (699:300:1–399:600:1, v/v) over 60 min. The flow rate was 1 ml/min and 2-ml fractions were collected.

3. RESULTS

Gel filtration of 5 human pancreas extracts gave variable profiles of GLP-1 and GLP-2 immunoreactivities. However, a common feature was a peak of K_{av} 0.15 containing both GLP-1 and GLP-2 immunoreactivities, with a smaller peak of GLP-1 immunoreactivity at K_{av} 0.43, the elution position of synthetic GLP-1 (fig.1A). In the K_{av} 0.15 peak, the ratio of GLP-1 to GLP-2 immunoreactivities was approx. 1:1 in 4 cases, but 1:0.3 in one case. HPLC (fig.2A) also demonstrated a peak (fractions 19–22) containing both GLP-1 and GLP-2 immunoreactivities, and an earlier peak (fractions 16–18) of GLP-1 immunoreactivity in the position of synthetic GLP-1

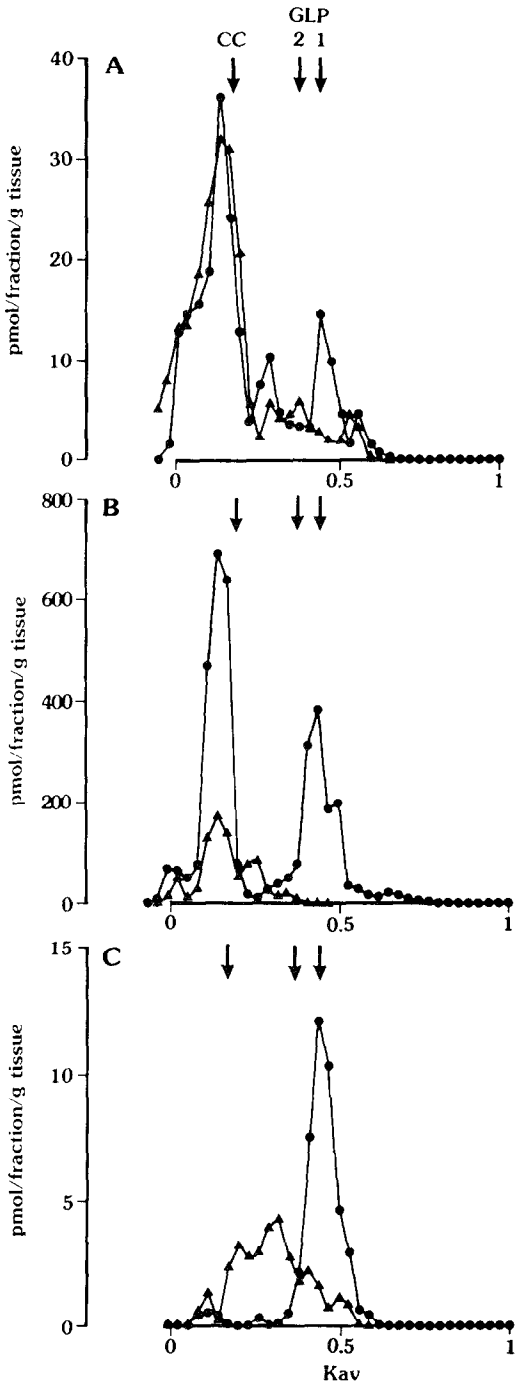

Fig.1. Sephadex G-50 chromatography of human tissue extracts analysed for GLP-1 (●) and GLP-2 (▲) immunoreactivities. (A) Pancreas, (B) glucagonoma, (C) ileum. Arrows show the elution positions of horse heart cytochrome *c* (CC) and synthetic GLP-1 and GLP-2 markers.

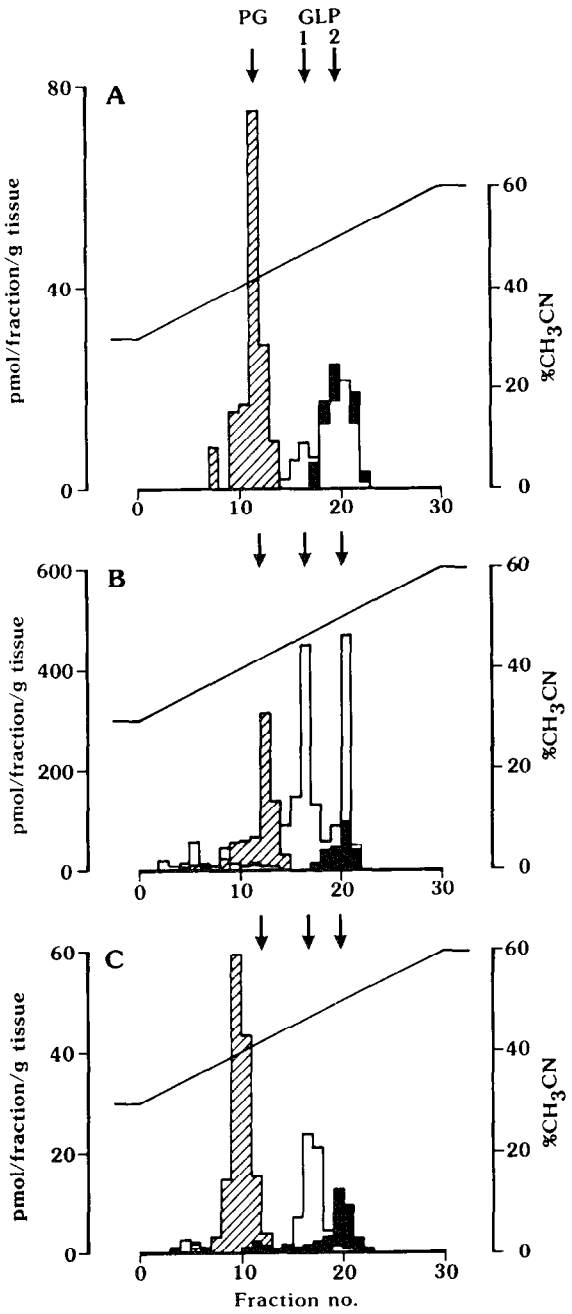

Fig.2. HPLC profiles of human tissue extracts analysed for glucagon-like immunoreaactivity (hatched bars), GLP-1 immunoreactivity (open bars) and GLP-2 immunoreactivity (solid bars). (A) Pancreas, (B) glucagonoma, (C) ileum. Arrows show the elution positions of pancreatic glucagon (PG) and synthetic GLP-1 and GLP-2 markers.

or des-Gly^{37}-GLP-1 amide, which were not separated in this system. GLI emerged as a peak of pancreatic glucagon (fractions 12–13), preceded by material that may correspond to N-terminally extended forms of pancreatic glucagon [8].

The pancreatic glucagonoma extract also contained a peak at K_{av} 0.15 with a GLP-1 to GLP-2 immunoreactivity ratio of 1:0.25, a small peak of GLP-2 immunoreactivity at K_{av} 0.23, and a substantial peak of GLP-1 immunoreactivity at K_{av} 0.43 (fig.1B). The corresponding HPLC profile showed a peak with GLP-1 and GLP-2 immunoreactivities (fractions 19–22), with a peak corresponding to free GLP-1 at fractions 16–18 (fig.2B).

By contrast, gel filtration of human ileal extract gave only a single peak of GLP-1 immunoreactivity at K_{av} 0.43, and fused peaks of GLP-2 immunoreactivity at K_{av} 0.20–0.31 (fig.1C). HPLC confirmed the presence of GLP-1 immunoreactivity emerging in the position of synthetic GLP-1, and showed a separate peak of GLP-2 immunoreactivity in the position of synthetic GLP-2 (fig.2C). However, the gel-filtration profile suggested that this must consist of at least 2 extended forms of GLP-2 that were not distinguishable from synthetic GLP-2 in this HPLC system. Similar patterns were obtained for human colon and rectum. Ratios of GLI:GLP-1:GLP-2 immunoreactivities recovered in the HPLC peaks were 100:42:22 for ileum, 100:47:8 for colon and 100:82:23 for rectum.

4. DISCUSSION

When these results are interpreted in the light of the deduced structure of human preproglucagon [1], it is apparent that the human pancreas consistently produces a proglucagon fragment that emerges before horse heart cytochrome *c* on gel filtration, contains both GLP-1 and GLP-2 immunoreactivities, but is devoid of GLI. This is compatible with the production of a cleavage product consisting of GLP-1 linked to GLP-2 via the intervening 'spacer' peptide. Pulse-chase studies on proglucagon biosynthesis in rat islets have demonstrated this fragment to be the principal or sole product from the C-terminal portion of rat proglucagon [9]. The situation in the human pancreas is more complex, as there is also some

production of free GLP-1, and an occasional deficit of GLP-2 immunoreactivity in the K_{av} 0.15 peak. This implies that there is some cleavage at the C-terminus of the GLP-1 sequence, and also some degradation or derivatisation within the sequence of GLP-2, reducing the GLP-2-like immunoreactivity in this peak. In the pancreatic glucagonoma there was also a deficit of GLP-2-like immunoreactivity within the K_{av} 0.15 peak, and a substantial amount of free GLP-1. It is possible that the GLP-2 immunoreactive peak at K_{av} 0.23 corresponds to GLP-2 linked to the spacer peptide at its N-terminus.

In the human intestine there is no peak with proportional GLP-1 and GLP-2 immunoreactivities, but there is a peak corresponding to free GLP-1 on both gel filtration and HPLC. There is also some separate GLP-2 immunoreactivity of greater molecular size than synthetic GLP-2; the deficit of this with respect to both GLI and GLP-1 immunoreactivity implies, as above, degradation or further post-translational modification within the GLP-2 sequence, or impaired cross-reactivity of possible N-terminally extended forms of GLP-2. The lesser deficit of GLP-1 may be similarly explained.

The occurrence of free GLP-1 in the intestine, combined with the demonstration that synthetic des-Gly^{37}-GLP-1 amide has a glucose-dependent insulinotropic effect on isolated rat islets [10], makes GLP-1 a candidate 'incretin', i.e. a candidate hormone increasing the insulin response to oral nutrient.

ACKNOWLEDGEMENTS

This work was supported by a studentship (S.K.G.) and a training fellowship (L.O.U.) from the Medical Research Council of Great Britain, and by a post-doctoral fellowship (M.G.) from the Spanish Ministry of Education and Science.

REFERENCES

[1] Bell, G.I., Sanchez-Pescador, R., Laybourn, P.J. and Najarian, R.C. (1983) Nature 304, 368–371.
[2] Heinrich, G., Gros, P. and Habener, J.F. (1984) J. Biol. Chem. 259, 14082–14087.
[3] Uttenthal, L.O., Ghiglione, M., George, S.K., Bishop, A.E., Polak, J.M. and Bloom, S.R. (1985) J. Clin. Endocrinol. Metab. 61, 472–479.
[4] O'Shaughnessy, D.J. (1982) in: Radioimmunoassay of Gut Regulatory Peptides (Bloom, S.R. and Long, R.G. eds) pp. 11–20, Saunders, London.
[5] Bryant, M.G. (1982) in: Radioimmunoassay of Gut Regulatory Peptides (Bloom, S.R. and Long, R.G. eds) pp.21–27, Saunders, London.
[6] Ghatei, M.A., Uttenthal, L.O., Bryant, M.G., Christofides, N.D., Moody, A.J. and Bloom, S.R. (1983) Endocrinology 112, 917–923.
[7] Laurent, T.C. and Killander, J. (1964) J. Chromatogr. 14, 317–330.
[8] Ghatei, M.A., Uttenthal, L.O., Christofides, N.D., Bryant, M.G. and Bloom, S.R. (1983) J. Clin. Endocrinol. Metab. 57, 488–495.
[9] Patzelt, C. and Schiltz, E. (1984) Proc. Natl. Acad. Sci. USA 81, 5007–5011.
[10] Schmidt, W.E., Siegel, E.G. and Creutzfeldt, W. (1984) Diabetologia 27, 328A.